

## APPARENT ABSENCE OF GAP JUNCTIONS IN TWO CLASSES OF CNIDARIA

G. O. MACKIE\*, P. A. V. ANDERSON†, AND C. L. SINGLA\*

† *C. V. Whitney Laboratory and Department of Physiology, University of Florida, St. Augustine, Florida 32086*, and \**Biology Department, University of Victoria, Victoria, British Columbia, Canada V8W 2Y2*

### ABSTRACT

Study of the literature and new observations by electron microscopy suggest that gap junctions are absent in the anthozoa and scyphozoa, but present in the hydrozoa. While this may help to explain the marked electrophysiological differences known to exist between the hydrozoa and the other two groups, it raises questions about how intercellular metabolic communication is achieved in the groups lacking gap junctions.

### DISCUSSION

In many tissues of metazoans from *Hydra* to the mammals, cell interiors are directly linked by aqueous channels represented structurally by the channels of gap junctional particles, or connexons (Unwin and Zamphighi, 1980). The diameter of the channel, determined by probing with fluorescent molecules, is estimated to be 16–20 Å in mammals and 20–30 Å in insects (Schwarzmann *et al.*, 1981). Gap junctions are widely believed to be responsible for electrical and dye coupling and for the transmission of electrical signals within various excitable tissues. While final proof is still lacking, gap junctions probably play an important role in tissue homeostasis by allowing permeant molecules to equilibrate throughout groups of coupled cells, in transport of nutrients from cell to cell, and in the dissemination of regulatory molecules (reviewed by Loewenstein, 1981). These regulatory functions are thought to be especially important in embryonic and differentiating tissues where gap junctions are frequently found, along with electrical coupling.

Despite the circumstantial nature of much of the evidence for metabolic cooperation in cells joined by gap junctions, there can be little question that the first appearance of gap junctions in early metazoans represented a major organizational advance. The fact that sponges remain at the cellular rather than the tissue level (Hyman, 1940) may be due in large part to their apparent “genetic incapacity to produce gap junctions” (Mackie, 1984).

The lowest metazoans to have gap junctions are the cnidarians, specifically members of the class Hydrozoa. Evidence from conventional transmission electron microscopy, lanthanum staining, and freeze fracture work shows these junctions to be structurally closely similar to those of higher animals (Hand and Gobel, 1972; King and Spencer, 1979). Gap junctions are present in electrically coupled glandular epithelium (Mackie, 1976), simple epithelia (Josephson and Schwab, 1974; Satterlie and Spencer, 1983), myoepithelia (Chain *et al.*, 1981; Satterlie and Spencer, 1983) and between certain (but not all) neurons (Spencer and Satterlie, 1980; Spencer, 1981). Dye coupling has been demonstrated in several of these cases.

The most obvious function for gap junctions in hydrozoans is as a pathway for impulse transmission both between coupled neurons and between the cells in electrically

excitable epithelia which provide the non-nervous conduction pathways which are such a striking feature of hydrozoans (reviewed by Anderson, 1980). Whether they serve a role in metabolic communication is as much an open question here as in other groups. Morphogenetic regulatory molecules have been identified in *Hydra* but it is still not known if they spread within the epithelia, within nerves, or extracellularly (reviewed by Bennett *et al.*, 1981).

Ever since the earliest days of electrical recording from cnidarians it has been clear that the hydrozoans stand sharply apart from the other cnidarians in their electrophysiological characteristics. Josephson (1974) characterizes the dichotomy as follows: "The anthozoans and scyphozoans examined have what might be termed conventional electrophysiology. Signals recorded with extracellular electrodes from conducting systems and muscles are small, generally well under 1 mv, and critically dependent on electrode placement. This is . . . what one would expect for activity in diffuse fibers in a nerve net or thin muscle sheets." In the hydrozoans, on the other hand, conducting systems produce "large electrical signals, typically 1–10 mv. The size of these potentials and their insensitivity to small changes in electrode position indicate that they are generated by blocks of electrogenic epithelia." The cubomedusae, sometimes treated as a fourth cnidarian class (Werner, 1975; Passano, 1982) exhibit electrophysiological responses of the scyphozoan-anthozoan type (Satterlie, 1979; Satterlie and Spencer, 1979).

How are we to account for the existence within one phylum of groups having such profoundly different electrical profiles? In considering this question, it struck us that while the hydrozoan ultrastructure literature is replete with reports of gap junctions, we could recall no such reports from other cnidarian groups. A survey of the literature and discussions with colleagues bears this out. No one to our knowledge has found gap junctions in any cnidarian outside the hydrozoa. Their absence, with few exceptions (*e.g.*, Anderson and Schwab, 1981) has excited no comment.

To satisfy ourselves that the lack of such reports does not simply reflect the use of differing techniques, we have examined tissues from various scyphozoans and anthozoans using a standard procedure (Singla, 1978) that has revealed gap junctions in many hydrozoans. The scyphozoan tissues examined were taken from the arms and tentacles of *Haliclystus steinegeri*, *Thaumatoscyphus atlanticus*, and the gonads of *Cyanea capillata* and *Rhopilema verrilli*. Developing embryos and planulae of *Cyanea* were also examined. For anthozoans, tentacles from the sea anemones *Aiptasia pulchella* and *Corynactis californica* were investigated. In none of these tissues were gap junctions observed.

Taking these findings at face value, we can immediately see how the electrophysiological differences between hydrozoans and other groups might arise. In hydrozoans, gap junctions would provide close coupling and ready spread of depolarizations within epithelia, whether as propagative events or as local potentials spreading decrementally from neuroeffector junctions. The simultaneous depolarization of such groups of cells would, as Josephson suggested, generate large extracellular signals. The lack of such spread would account for the "conventional electrophysiology" of other cnidarians.

There is no evidence for electrical coupling between cells in anthozoans or scyphozoans. Intracellular recordings from one scyphozoan nerve net, the motor nerve net of *Cyanea capillata*, indicate that there is no coupling between the neurons. Instead, the synapses appear to be chemical (Anderson, unpub.). It has been suggested that the slow conduction systems (SS 1, SS 2) of corals and sea anemones such as *Calliactis* (reviewed by McFarlane, 1982) are neuroid systems of the hydrozoan type, and Shelton (1975) developed a computer model for the SS 1 based on the assumption of electrical coupling in the ectoderm, but none of the workers in this field would

claim that there is any direct evidence for coupling or even for the involvement of the epithelia as the slow conduction pathways.

The failure to observe gap junctions in these groups could merely mean that the junctions are very small, consisting of isolated connexons, or small groups of them. If this were so, the membranes of adjacent cells should show frequent close contacts. Study of the material does not support such a picture.

Alternatively, junctions other than gap junctions (*e.g.*, septate junctions) might provide for electrical communication between cells. Certainly such a possibility cannot be excluded *a priori*, but the available evidence is most easily explained on the assumptions that gap junctions are absent and that coupling, if it exists, must be very loose.

We come then to the hypothesis, which is also a conclusion from existing data, that among the Cnidaria only the hydrozoans have gap junctions. Many questions inevitably arise. Did the common ancestor of the Cnidaria have gap junctions, which survive only in the one class? If so, what led to their elimination in the other classes? If on the other hand the common ancestor lacked gap junctions and they were a hydrozoan invention, does this establish a hydrozoan as ancestor for all higher metazoa?

The gap junction is firmly established as a pathway for electrical communication and, in many cases, transmission of impulses, but what of its supposed role in metabolic communication? Despite their apparent lack of gap junctions the scyphozoans and the anthozoans are no less well organized histologically than the hydrozoans. Presumably, in the absence of direct pathways between cells, tissue communication could still be achieved by interaction of signaling molecules embedded in the membranes of adjacent cells, or by humoral signaling between the cells composing the epithelia (Loewenstein, 1984). Or, finally, tissue regulation could be achieved indirectly by trophic influences from nerves, as in the maintenance of vertebrate skeletal muscle (reviewed by Dennis, 1981). Nutrient transport could be largely extracellular, or could involve amoebocytes. These cells are present in the anthozoa and scyphozoa but are absent in hydrozoans, though in the latter interstitial cells are believed to assume some of the same functions (Chapman, 1974).

Any useful hypothesis should suggest experiments by which it can be tested. The obvious need highlighted by the arguments presented here is for verification of the two fundamental propositions, namely that gap junctions are truly absent from the tissues of scyphozoans and anthozoans and that their cells consequently have little if any capability for *direct* electrical or metabolic communication. If these propositions prove to be true, we will be in a much better position to explain the electrophysiological dichotomy that exists in the phylum, and to plan experiments which might elucidate the mechanisms of metabolic communication within the Cnidaria.

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